X-ray Fluorescence Microscopy for detection of nanoparticulate metal oxides and metals in mammalian cells

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Nanomaterials in Biology and Medicine

Nanomaterials in Biomedicine:

- Size < 100nm
- New qualitative properties on nanoscale

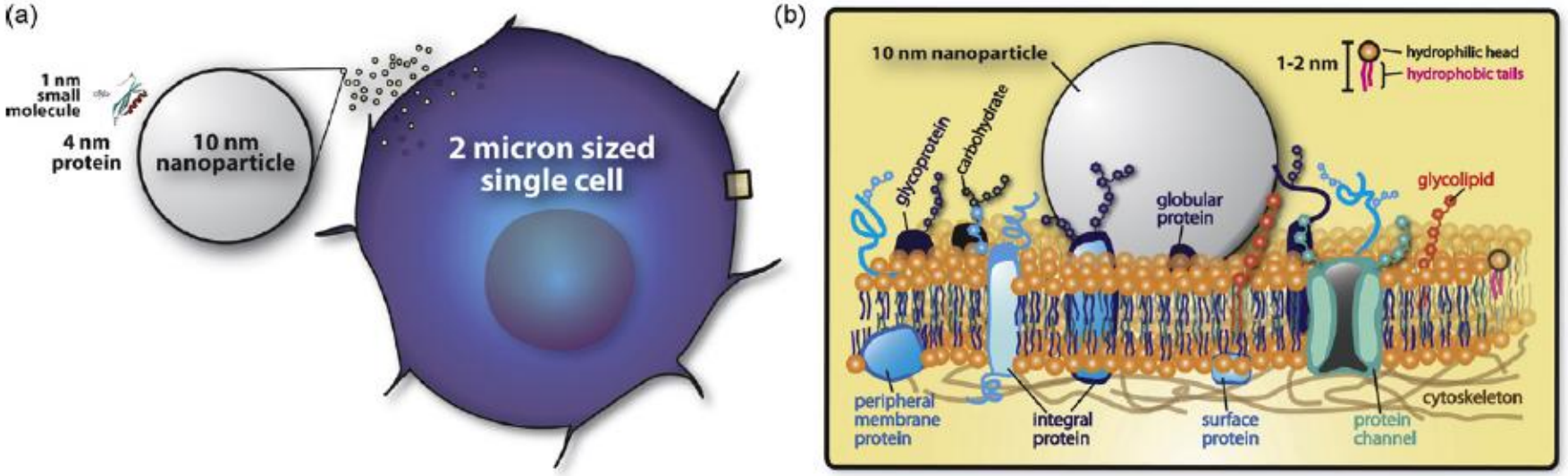
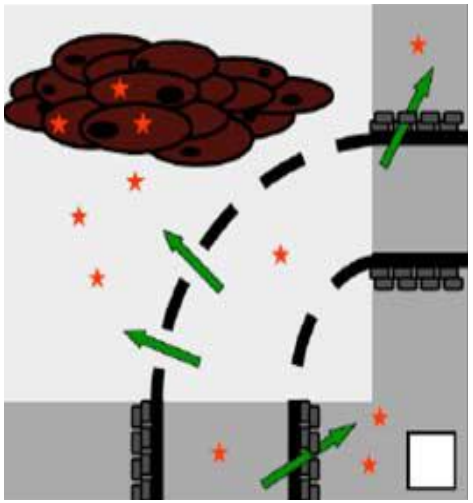


Fig. 2. Size matters. (a) Compared to a 10 nm nanoparticle, proteins (e.g. APP; X-ray crystal structure obtained from www.pdb.org (Berman et al., 2000), protein ID 2FKL; visualization done by Accelrys Discovery Studio Visualization 1.7 software) and small molecules (e.g. DHED) are small in size and volume. A mammalian cell which is made up of proteins, nucleic acids, and other small to large molecules is thousand times larger in volume and size compared to a 10 nm nanoparticle. (b) Cell membrane incorporating various proteins and a single 10 nm nanoparticle.

WH Suh, et al., Progress in Neurobiology 87 (2009) 133–170

Nanomaterials in Biology and Medicine—Cell Interactions *in vivo*

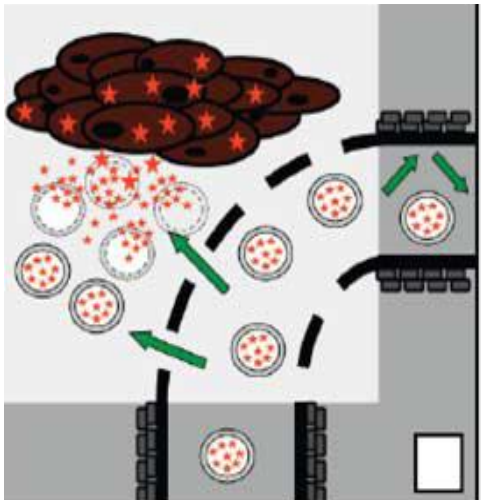


Current Chemotherapeutics

Non-specific biodistribution

Unwanted effects in normal tissues

Low level accumulation in tumor

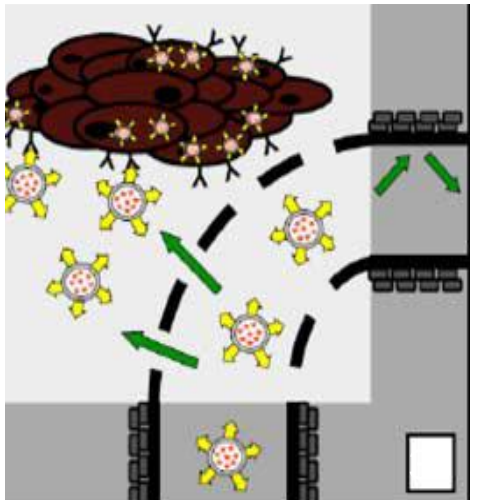


Passive Targeting to Tumor

Enhanced Permeability and Retention (EPR)

Increased drug load to tumor site

Decreased drug delivery to normal tissues



Active Targeting to Tumor

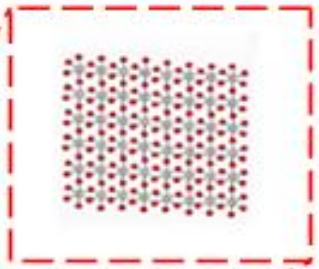
Over-expressed Surface Receptors

Increased efficacy due to greater internalization

Decreased drug delivery to normal tissues?

Lammers et. al. *British Journal of Cancer, 2008.*

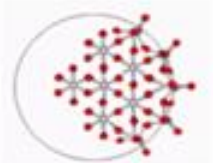
Bulk TiO_2 vs. Nanoscale TiO_2: Surface Properties of TiO_2 nanoparticles



Bulk

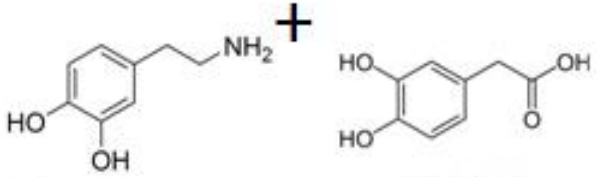
Octahedral

< 20nm

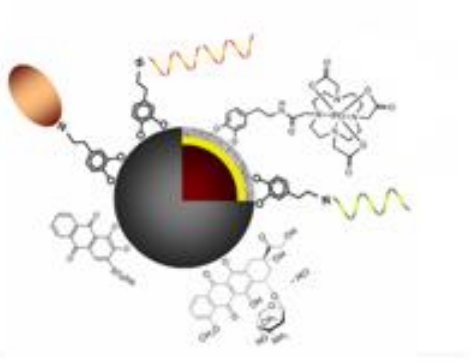


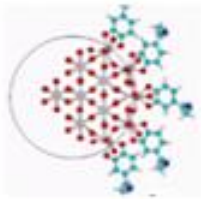
Nanoparticles (NPs)

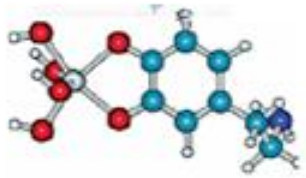
Square Pyramidal



Dopamine DOPAC





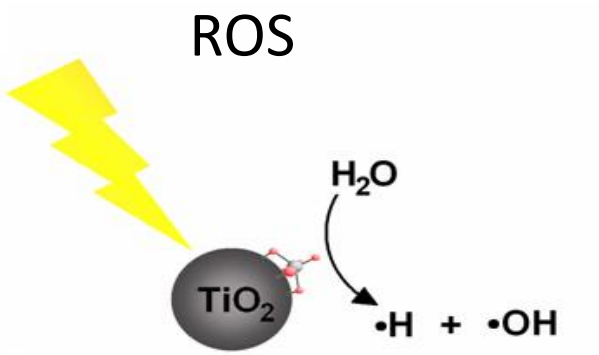


Nanoconjugates (NCs)

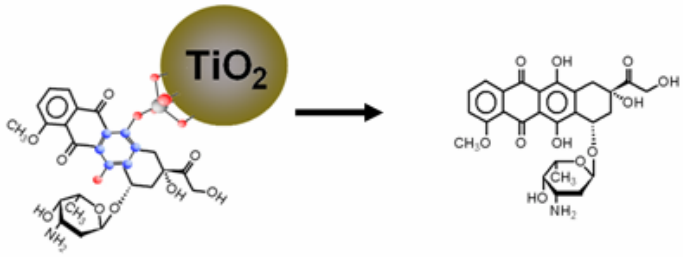
Octahedral

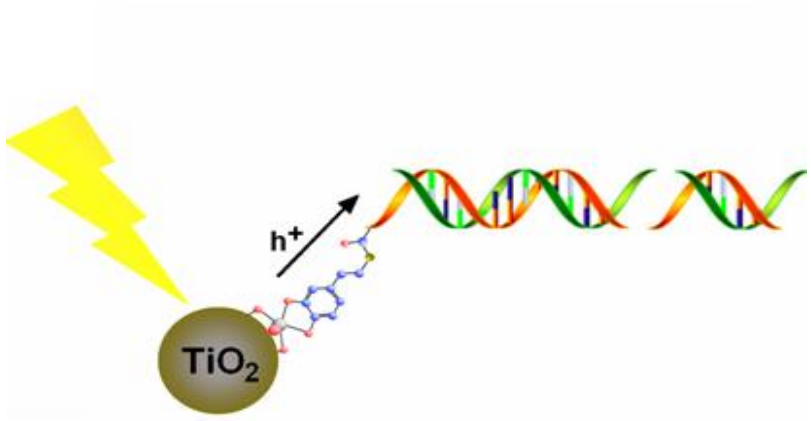
Four Possible Cytotoxic Mechanisms of Action of TiO_2 Nanoparticles and Nanoconjugates/Nanocomposites

ROS

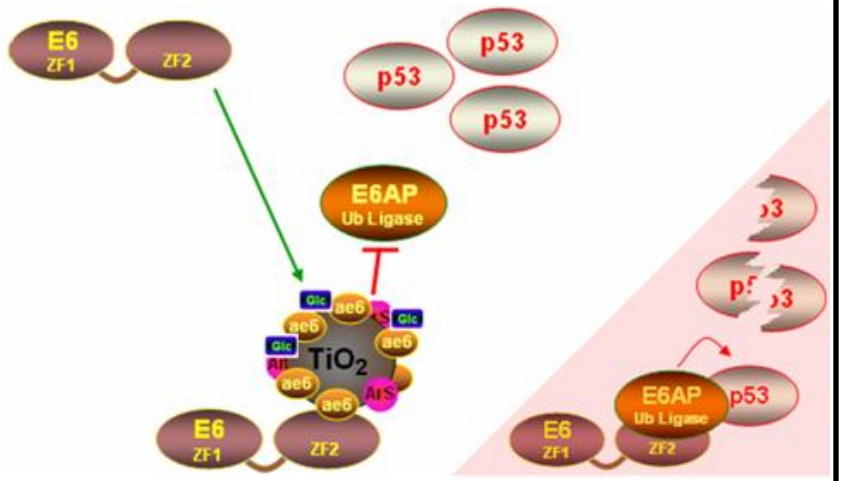


drug delivery + ROS





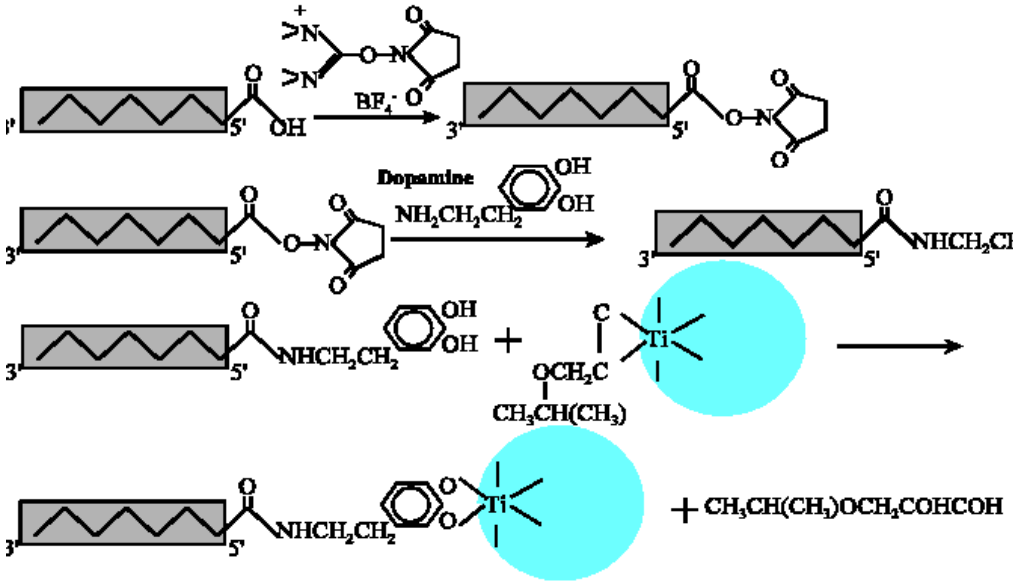
charge injection

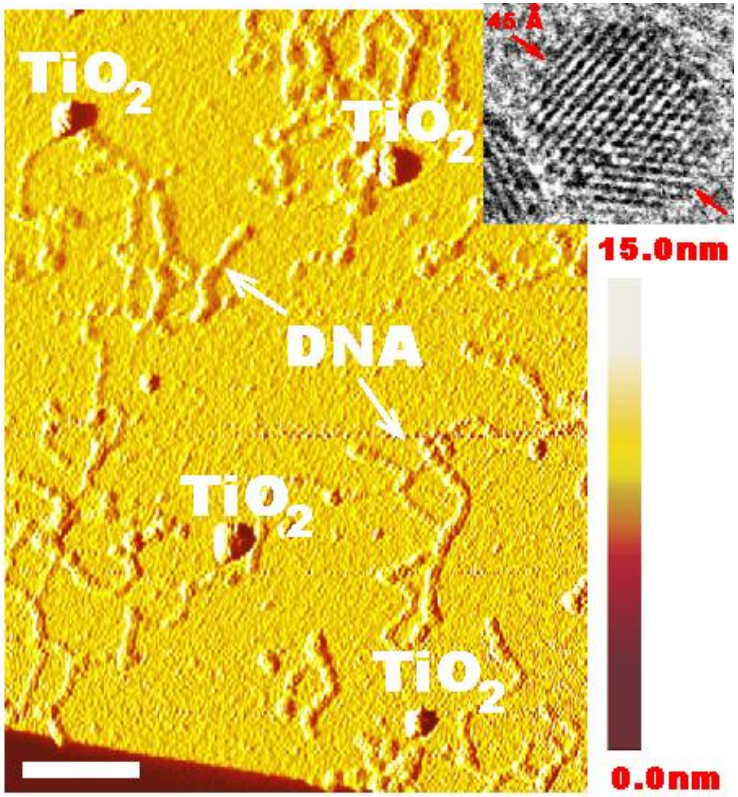


oncogene pathway interference

TiO_2–DNA nanoconjugates

DNA can be attached to the dopamine and serve as an organic nanoparticle surface modifier.





Atomic Force Microscopy image of TiO_2-oligonucleotide nanocomposites complementary to the lambda phage DNA following hybridization.
White scale bar presents 10 microns. Color gradient presents height.

In the upper right corner is a Transmission Electron Micrograph of a single TiO_2 particle showing its size (45 Å).

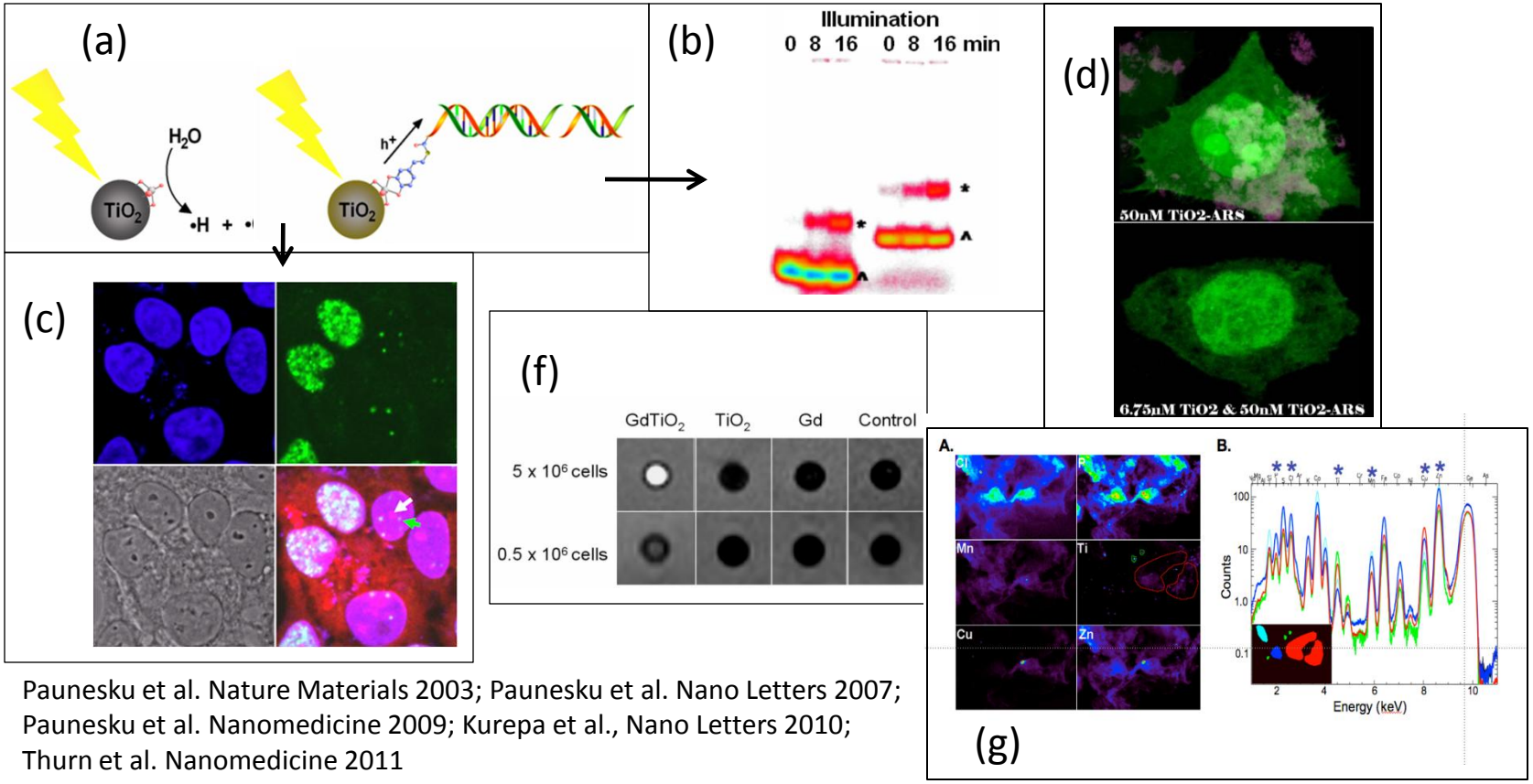
Paunesku T, Rajh T, Wiederrecht G, Maser J, Vogt S, Stojićević N, Protić M, Lai B, Oryhon J, Thurnauer M, Woloschak G. ***Nature Materials*** (2003) 2, 343-346

New technologies that will benefit from BioNanoprobe: Bionanotechnology

Bionanotechnology is providing new insights into basic science, its uses are increasing in medicine and associated sciences, while engineering of new bio-nano materials occupies much of the most visible government funded research and high impact publications.

Nanotechnology examples: TiO2 nanoparticles cause DNA damage by charge injection or production of reactive oxygen species, similar to the action of irradiation; moreover they can be used for fluorescence imaging and to improve contrast in medical imaging.

Example of funding agencies interest: NIH R25 Training Program: Funded by NCI in 2009 in anticipation of a nanotechnology-based transformation in the practice of tumor imaging and radiotherapy. This program has strong links to Northwestern's **Center for Cancer Nanotechnology Excellence**.

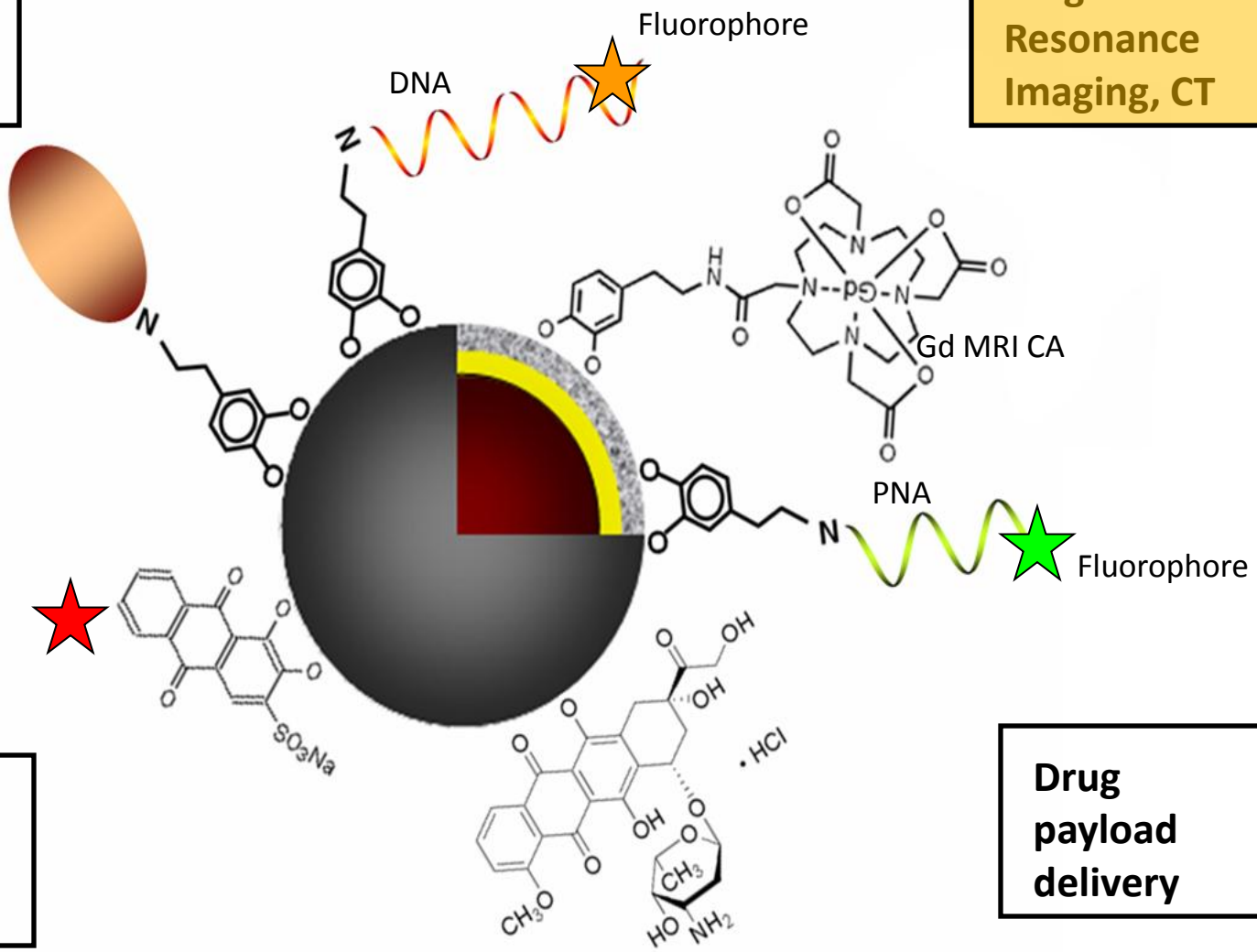


Paunesku et al. Nature Materials 2003; Paunesku et al. Nano Letters 2007; Paunesku et al. Nanomedicine 2009; Kurepa et al., Nano Letters 2010; Thurn et al. Nanomedicine 2011

Multifunctional TiO_2 Based Nanoconjugates

Targeting

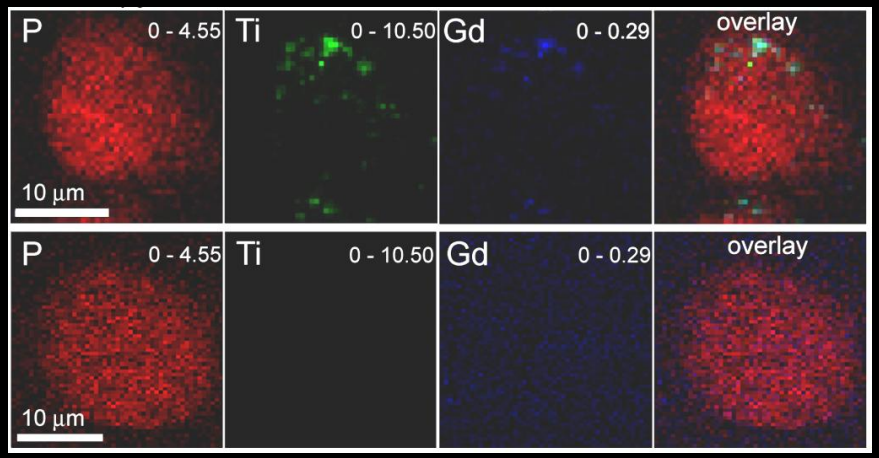
Magnetic Resonance Imaging, CT

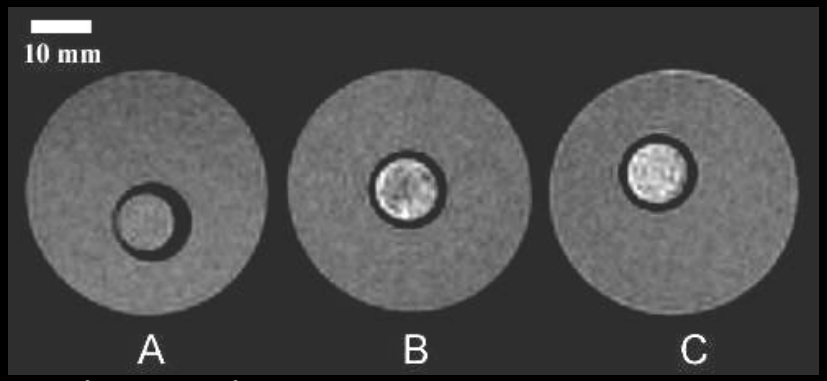


Optical imaging

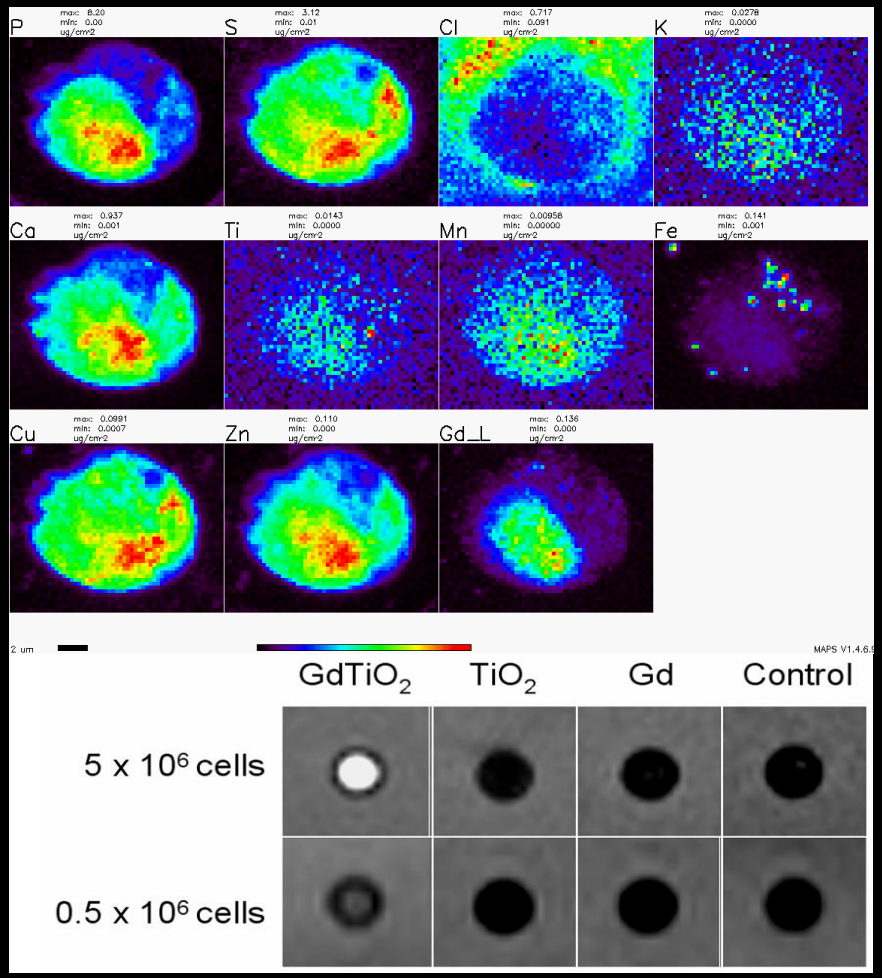
Drug payload delivery

Nanoparticles can be used as vehicles for delivery of Gd MRI contrast agents





Endres, et al. JACS 2007

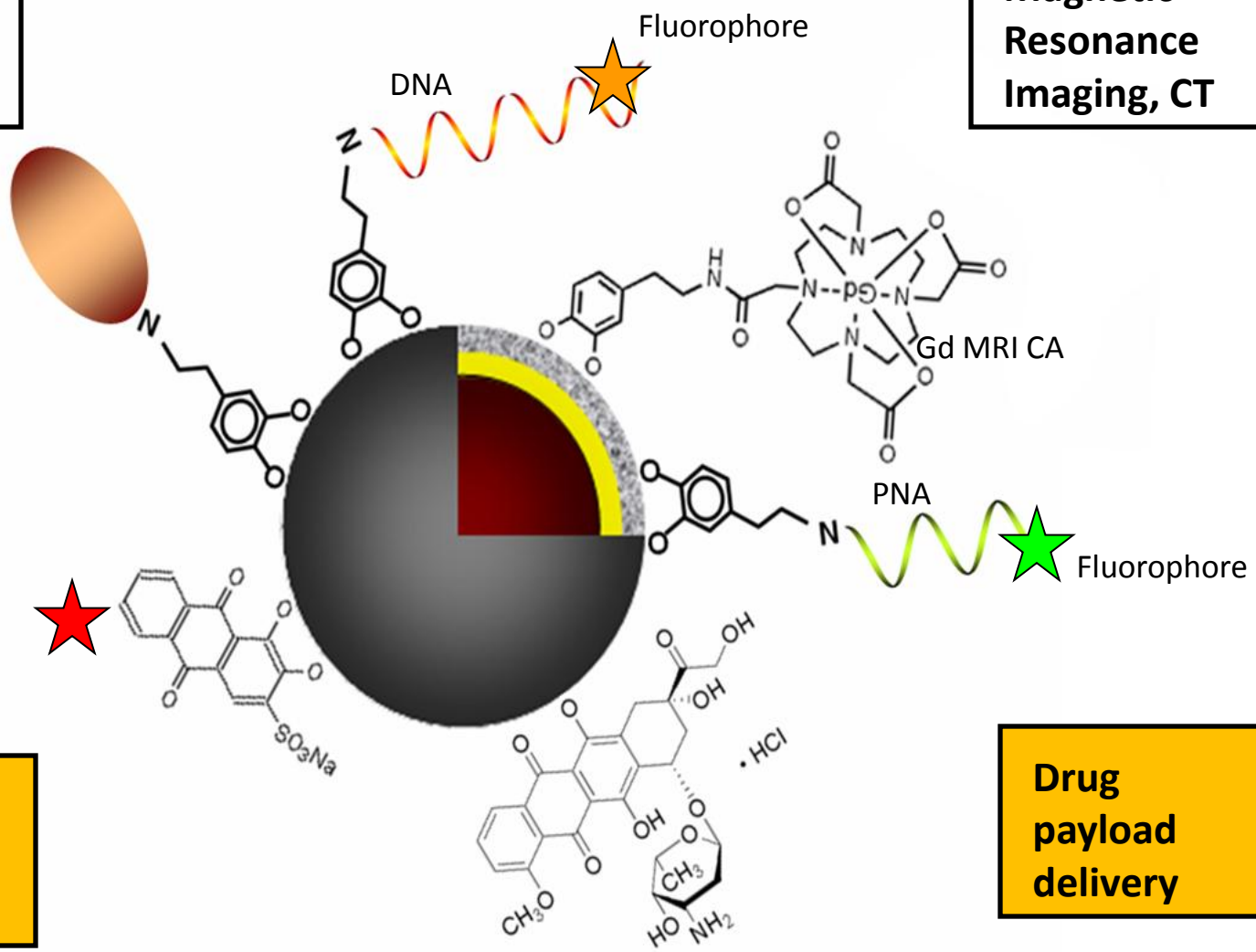


Paunesku, et al., Nanomedicine 2008

Multifunctional TiO_2 Based Nanoconjugates

Targeting

Magnetic Resonance Imaging, CT

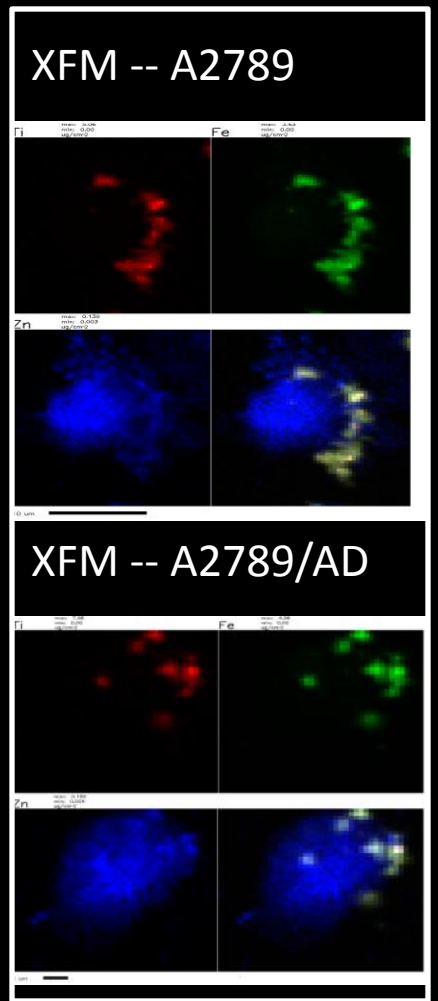


Optical imaging

Drug payload delivery

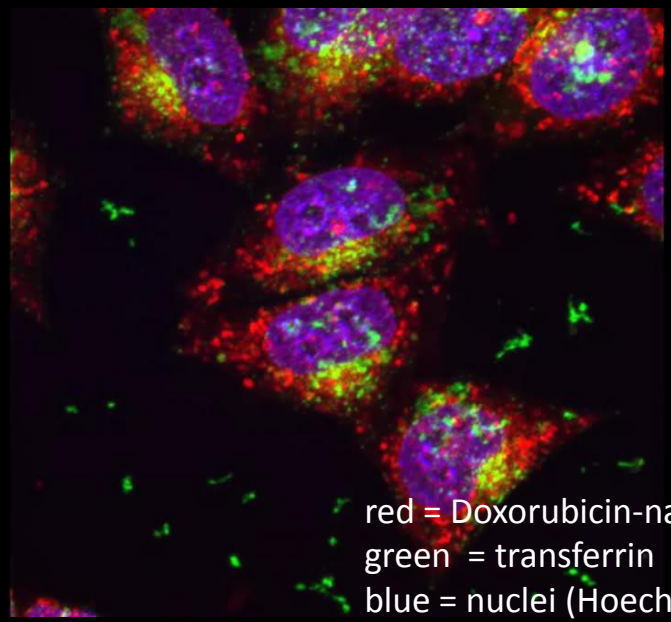
TiO_2 nanoparticles and nanocomposites can be used as vehicles for delivery of drug payloads

Cell lines resistant to small molecule formulations of different drugs may not be equally resistant to delivery of these molecules once they are conjugated to the nanocomposite surface. Doxorubicin conjugated to Fe3O4@TiO2 nanocomposites evades export proteins in doxorubicin resistant ovarian cancer cell lines. (Arora et al. 2012. Cancer Research)

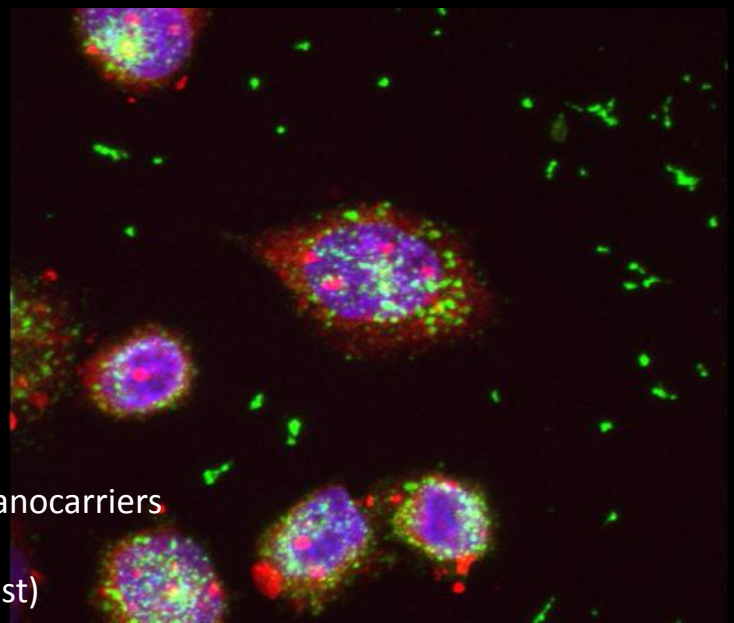


While confocal fluorescence "sees" only doxorubicin, X-ray fluorescence microscopy (XFM) "sees" only nanomaterial!

Doxorubicin-nanocarriers and transferrin at **2h** in a **doxorubicin sensitive cell line** A2789

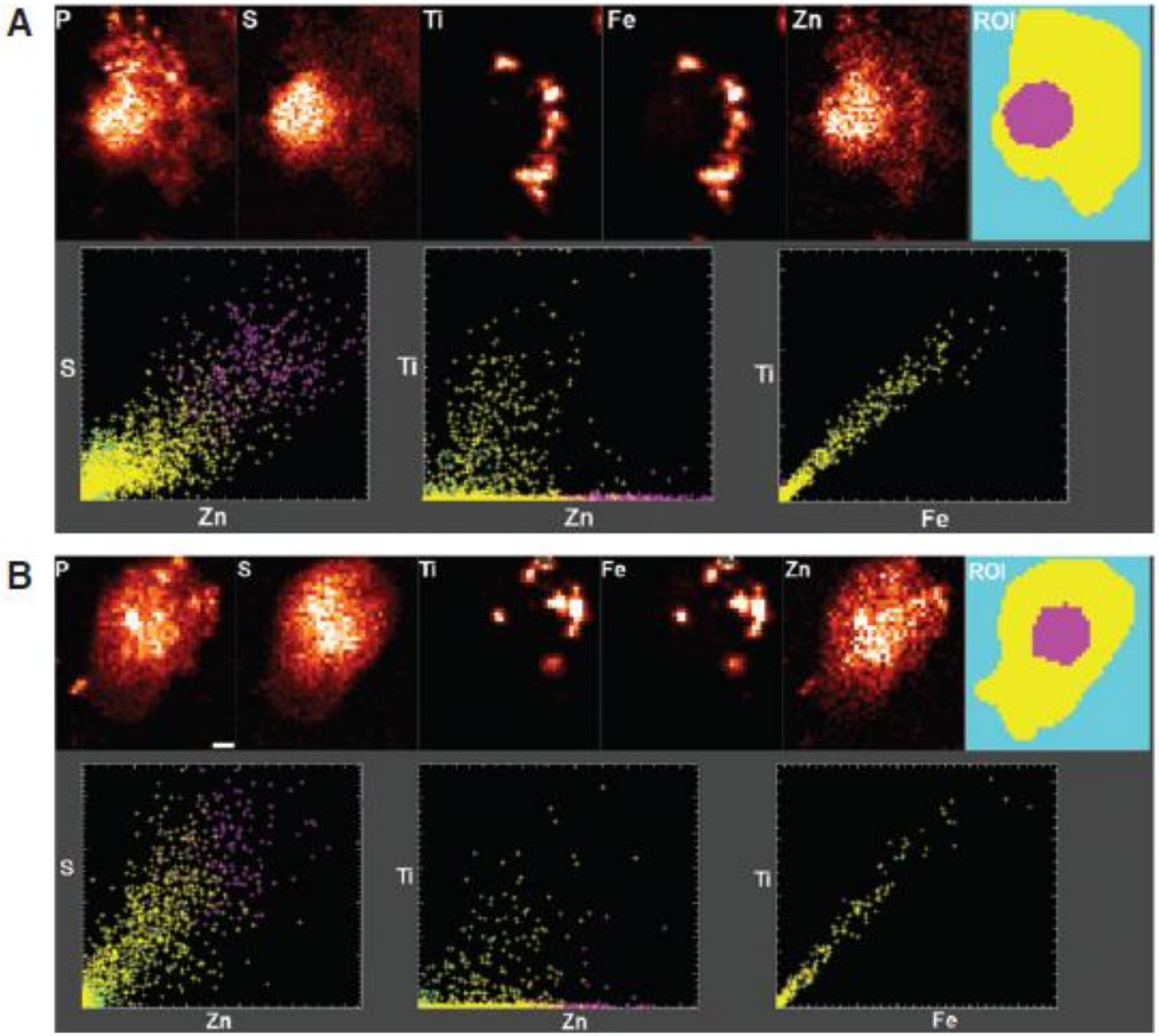


Doxorubicin-nanocarriers and transferrin at **2h** in a **doxorubicin resistant cell line** A2789/AD



red = Doxorubicin-nanocarriers
green = transferrin
blue = nuclei (Hoechst)

TiO_2 nanoparticles and nanocomposites can be used as vehicles for delivery of drug payloads



Ovarian cancer cell lines resistant to doxorubicin are not equally resistant to nanoparticles.

XFM shows that nanoparticle distribution patters is the same in both cell lines.

Doxorubicin sensitive (A) and resistant (B) cells were treated with nanoparticles. Elemental fluorescence for P, S, Ti, Fe, and Zn is shown as a red temperature false color image with black as the lowest and white as the highest signal.

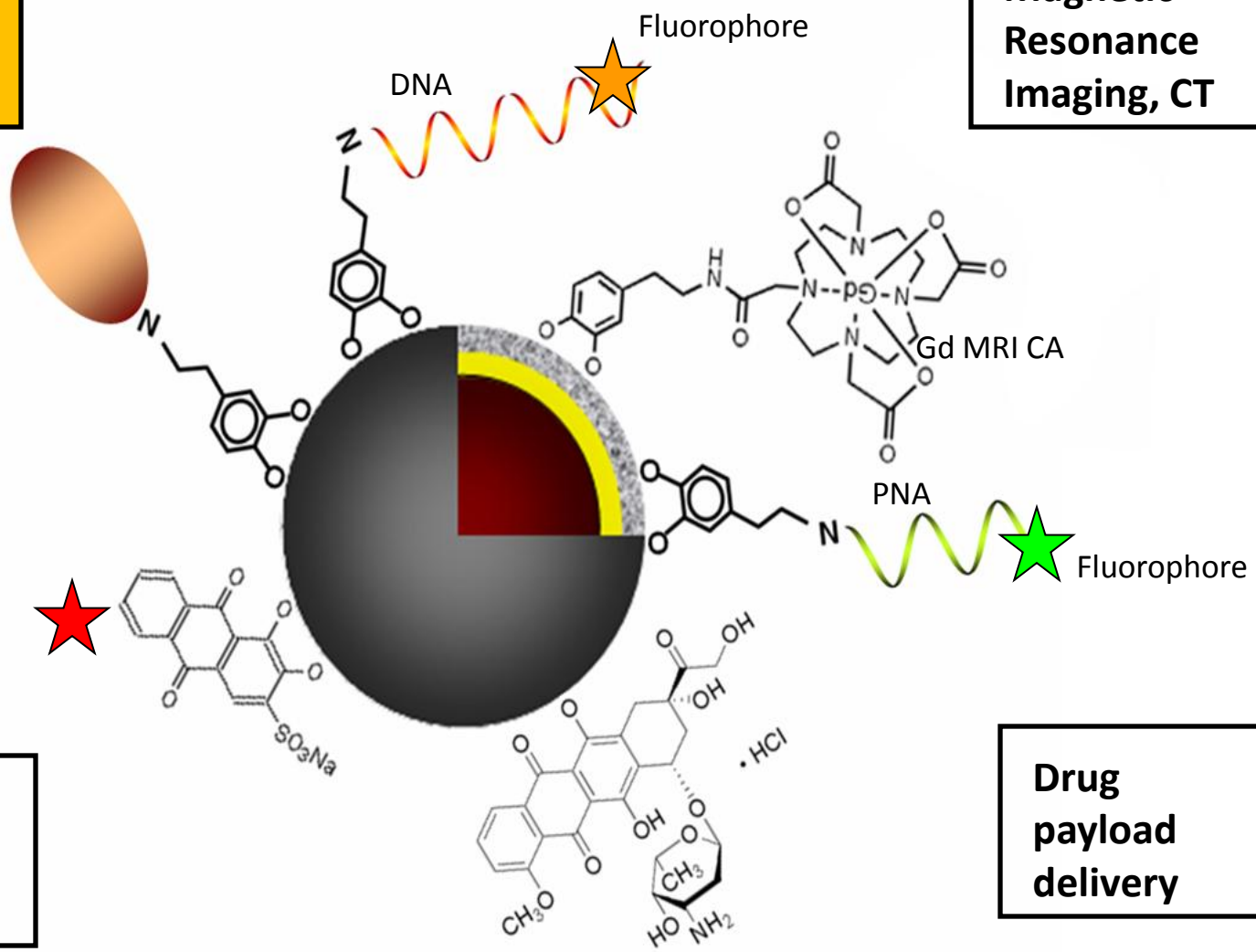
TitaniumK a fluorescence, indicating the location of the nanocomposites is notable only in the cytoplasm of both cell lines in a vesicle pattern; iron distribution is follows the same pattern. Fluorescence of sulfur indicates the cell outline, the highest Zn signal shows the location of the nucleus. The cell diagram with 3 regions of interest: cell nucleus (pink), cytoplasm (yellow), and background (light blue) matches scatter plots.

Arora et al. 2012. Cancer Research

Multifunctional TiO_2 Based Nanoconjugates

Targeting

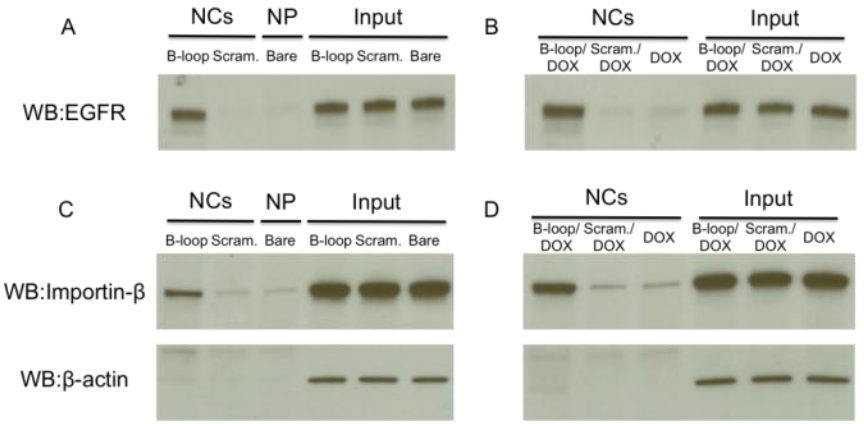
Magnetic Resonance Imaging, CT



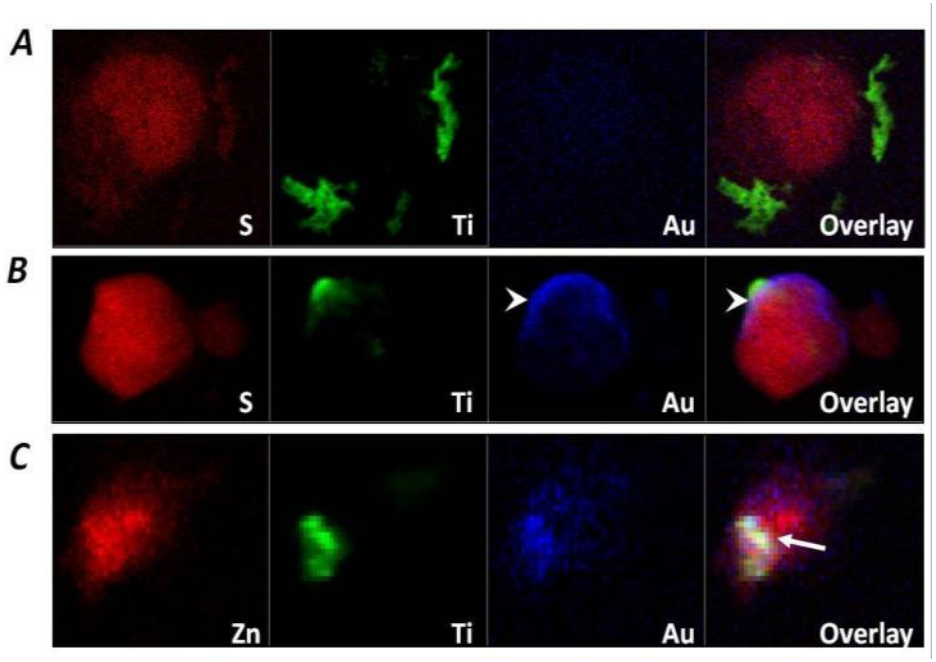
Optical imaging

Drug payload delivery

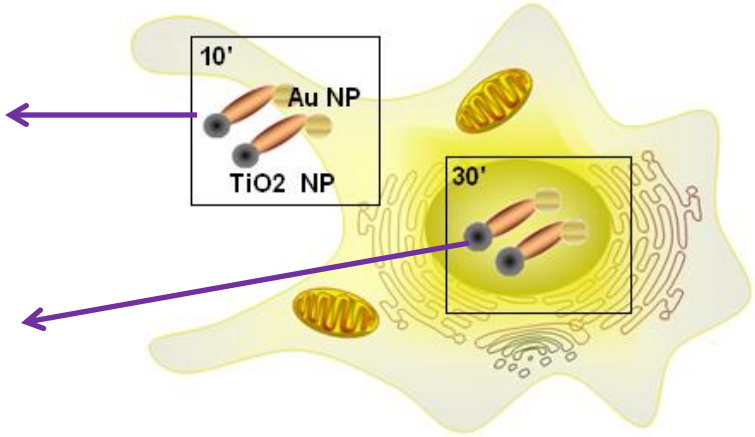
Nanoparticles Targeted to Sub-cellular Locations



Carefully designed peptide tagged nanoparticles behave similar to natural protein ligands, in complex protein mixtures and in cells *in vitro*.
Yuan et al (submitted)



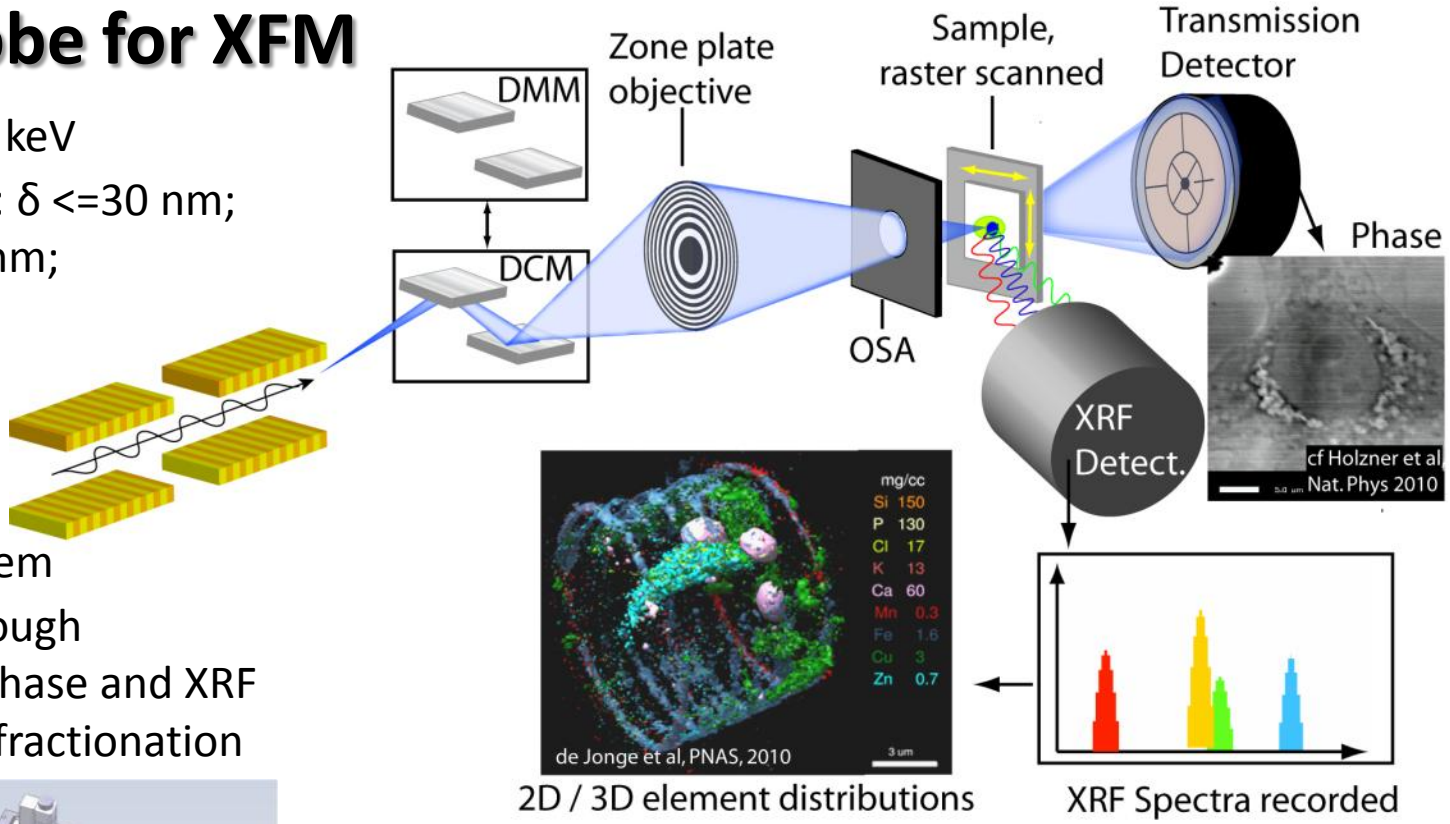
Non-targeted (A) and peptide targeted nanoconjugates at 10' (cell surface) (B) and 30' (cell nucleus and cytoplasm) (C) (Yuan et al 2011)



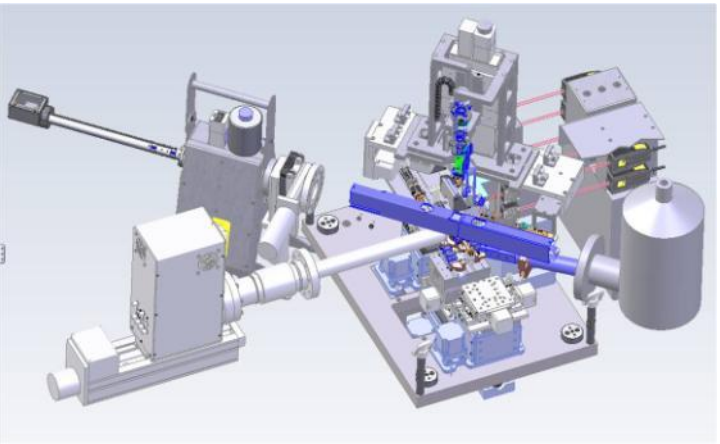
Detection of Nanoparticles by HIGH Resolution XFM: Subcellular Targeting

Bionanoprobe for XFM

- Energy range: 5 – 20 keV
- resolution: Mapping: δ <=30 nm; Spectroscopy: δ =50 nm;



- in vacuum, cryo-system
- fast tomography through combination of diff. phase and XRF mapping, using dose fractionation



NIH/NCRR funding (PI: G. Woloschak, Northwestern University). BioNanoprobe will be built by Xradia, installed at APS: LS-CAT (sector 21), commissioning: summer 2011

co-Investigators:
S. Vogt (tech lead), T. Paunesku, K. Brister (LS-CAT tech lead), B. Lai, J. Maser, C. Jacobsen, W. Anderson, *et al*

Argonne NATIONAL LABORATORY

NORTHWESTERN UNIVERSITY FEINBERG SCHOOL OF MEDICINE

xradia insight in 3D

Conclusions From Initial Bionanoprobe Studies

- Great depth of focus allows successful imaging of 10 um thick samples in 3D
- 3D XFM images of cells treated with nanoparticles of non-biological makeup give complete uptake information—nanoparticle subcellular location, quantity etc
- Flash freezing followed by cryogenic sample processing allows native cell imaging (w/o stains) of whole cells at resolution close to TEM

Questions for the SRX meeting participants:

If you think about the unique capabilities of SRX, what experiment comes immediately to your mind that you would like to do?

Imaging nanoparticle uptake in large tissue samples with different resolutions and high throughput.

How should the sample environment look like? What would be the demands on the environment from your samples?

Ability to use large, centimeter sample sizes is most important. They could be dry/embedded although ability to use frozen samples and cryo scanning would be additional advantage.

Which elements with absorption edges in the energy range covered by SRX (4.65keV to 22keV) would be of most interest for you?

Biologically relevant elements (P through Zn) are necessary to define samples. Other elements that can be used for “staining” (Br, I, etc) are also of importance. Nanomaterials elemental components need to be imaged w/o the need for staining, therefore nanomaterial ingredients Ti, Fe, Au need to be identifiable by XFM.

What would be more important for you for an experiment at SRX, very high spatial resolution (sub-100nm) or a large sample area (mm with sub-micron resolution)? Would a "zooming-in" capability be of interest?

Zooming is would be very useful, espec. because X-rays setup could (at least in theory) remain the same.

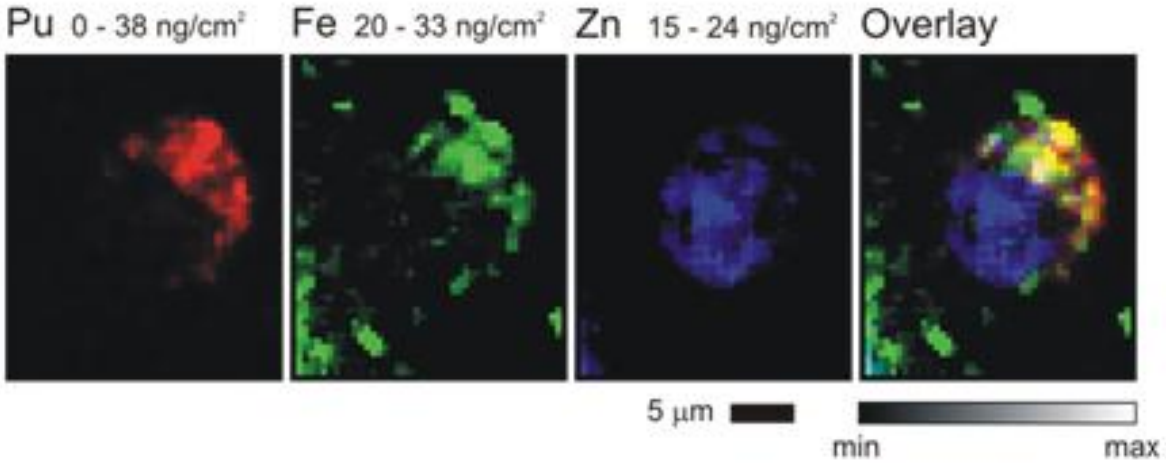
Which of the techniques available at the SRX beamline would be absolutely crucial for your experiment?

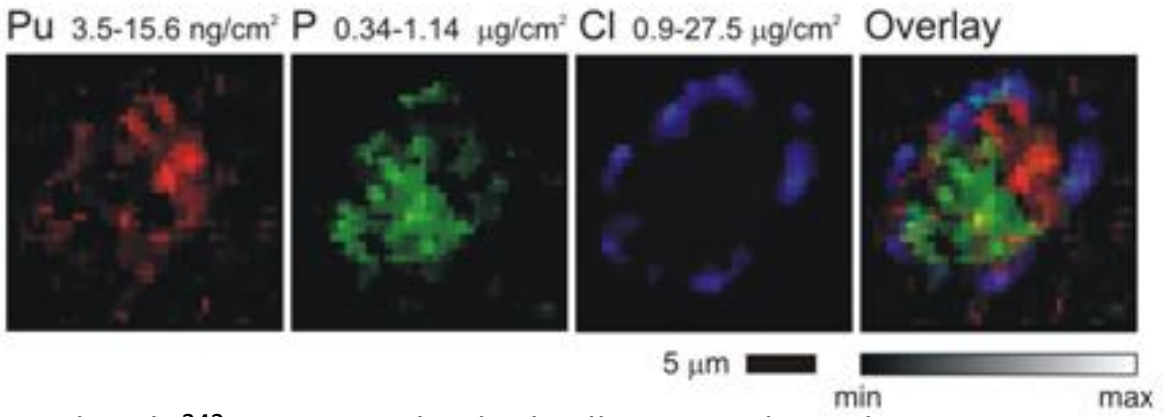
XFM for P-Zn

XANES for Ti, Fe, other potential NP components

XFM and XANES for plutonium studies

Pu is localized in cytoplasm—overlap with Fe?





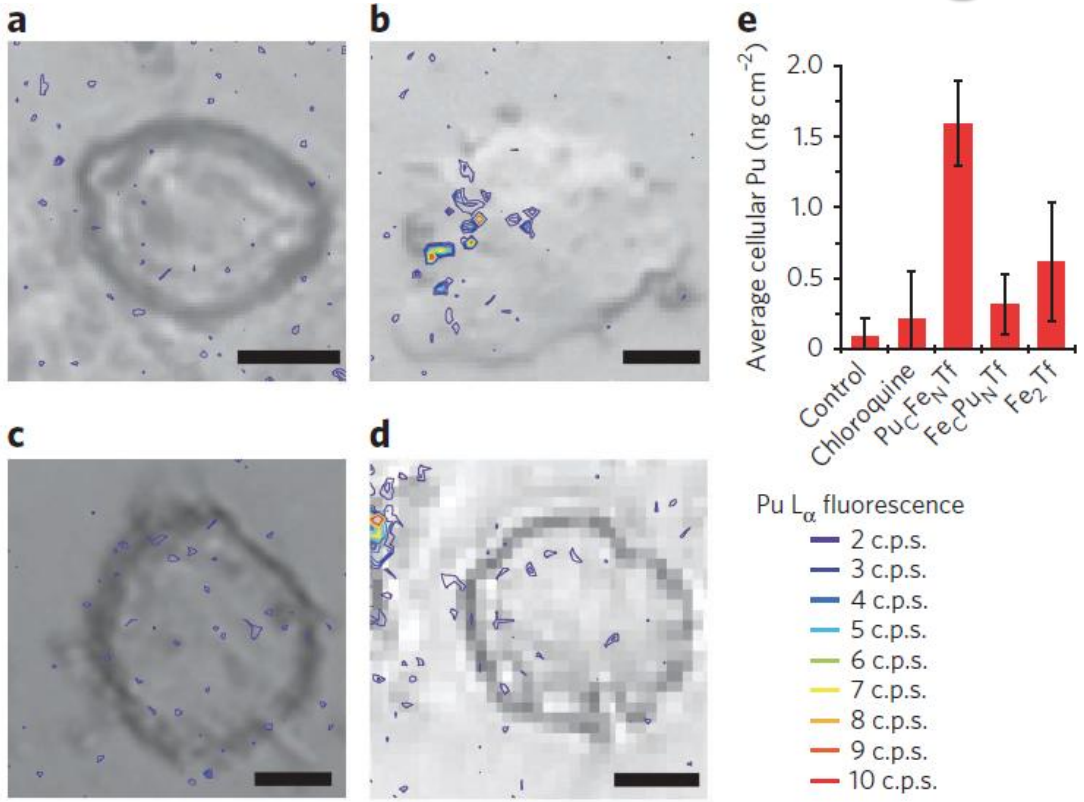
PC12 cells were treated with ^{242}Pu. Two individual cell images show that:

(1) Pu (red) and Fe (green) co-localize (yellow) --- initial hypothesis that Fe and Pu enter cell by the same mechanism

(2) Pu (red) and Zn in upper cell (blue) or P (green) in lower cell do not co-localize --- since P and Zn are the highest in the nucleus, Pu apparently remains in the cytoplasm

Jensen et al., 2011, Nature Chemistry

Pu and Fe enter cells using transferrin



Contour plots of Pu L lines X-ray fluorescence superimposed on optical images of cells:

(a) Pu-free control cell

(b) uptake of Pu as Pu_CFe_NTf

(c) very low uptake of Pu as Fe_CPu_NTf

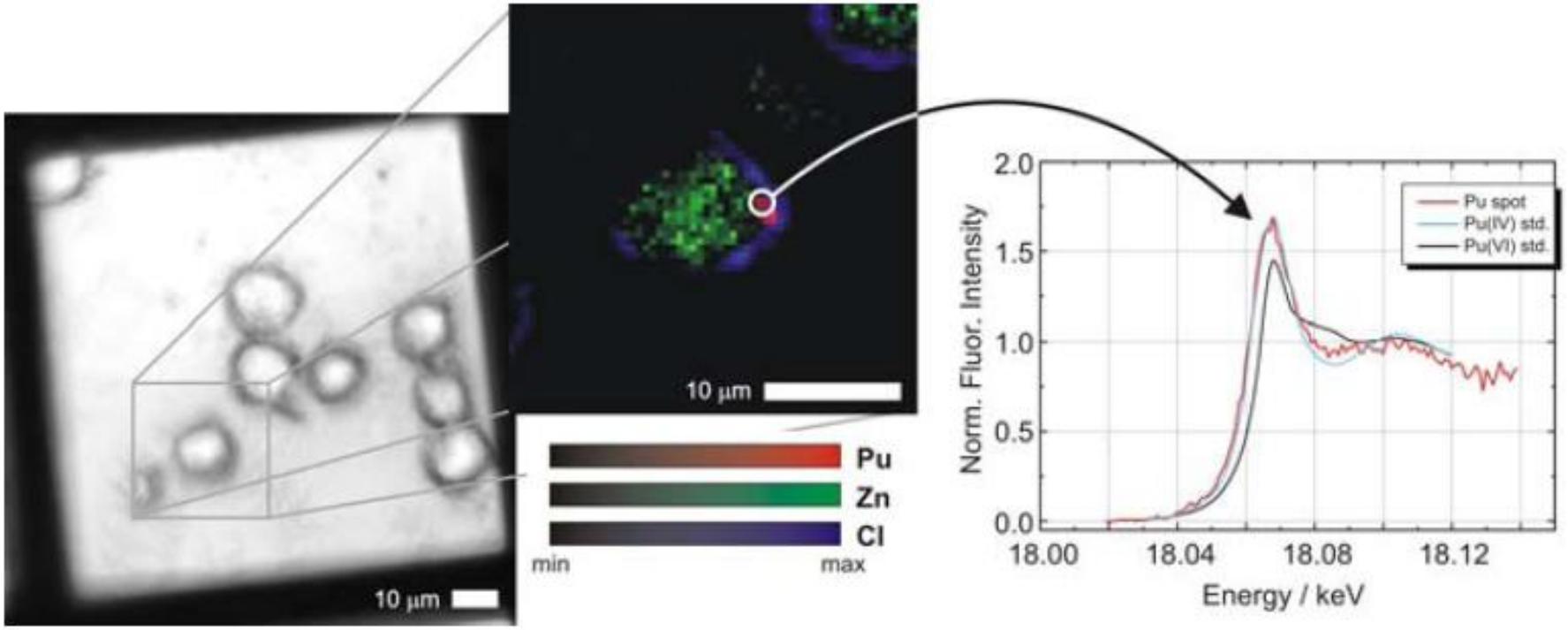
(d) very low uptake of Pu as Pu_CFe_NTf in chloroquine-treated cells exposed

Scale bars, 5 µm.

(e) The average background-corrected Pu content of the Pu-containing cells exposed to Pu as Pu_CFe_NTf alone (n = 8) or with 50 µM chloroquine (n=8) or competed with Fe_2Tf (n=4). The error bars are ± s.e.m. at 95% confidence level.

Jensen et al., 2011, Nature Chemistry

Pu X-ray absorption spectroscopy—only tetravalent Pu is inside cells



Pu was added to the media of PC12 cells in different oxidation states: Pu(III), Pu(IV), and Pu(VI) and in different chemical forms.

Regardless of the initial oxidation state or chemical form of Pu presented to the cells, only tetravalent Pu was found in the cytoplasm.

Gorman-Lewis et al., 2011 Inorganic Chemistry

Use of X-rays for cellular Pu studies

- In rat pheochromocytoma PC12 cells treated with isotope ^{242}Pu XFM was used to image and quantify plutonium at the L_3 or L_2 edge :
  - Pu was localized principally in the cytoplasm overlapping with Fe;
  - Pu detection limit was 1.4 fg Pu/cell or 2.9 x 10^{-20} moles Pu/μm^2,
  - Pu that can be found intracellularly is always tetravalent, regardless of the fact that Pu added to the media was in different oxidation states (Pu(III), Pu(IV), and Pu(VI)).
- SAX analysis has shown that only Pu_CFe_NTf mimics Fe_2Tf and binds to TR to be endocytosed

Collaborators

- Northwestern University:
  - Radiology: Reed Omary, Debiao Li, Nicole Mascheri, Andy Larson, et al.
  - Pharmacology: Ray Bergan
  - Urology: Chung Lee
  - Material Sciences: Vinayak Dravid, Mohammed Aslam
  - Chemistry: Tom Meade, Paul Endres
  - CIF: Teng Leong Chew
- ANL:
  - Chemistry: Mark Jensen, Lynne Soderholm
- APS:
  - Bio-CAT: Tom Irving, Raul Barrea
  - XOR: Stefan Vogt, Barry Lai, Jorg Maser, Si Chen, Chris Jacobson
  - LS-CAT/Bionanoprobe: Keith Briester, Wayne Anderson
- UIC:
  - Biomedical Engineering: Andreas Linninger
  - Pharmacy: Dejan Nikolic
- Case Western:
  - Chemistry: Zheng-Rong Lu
- Duke University:
  - Microbiology: Ann Lefurgey, Peter Ingram
- University of California Davies:
  - Radiation Oncology: JianJian Li
- University of Chicago:
  - Radiology: Gregory Karczmar, Sunny Jansen
  - Radiation Oncology: David Grdina, Jeff Murley
- University of Kentucky:
  - Plant Biology: Jasmina Kurepa, Jan Smale
- Ningbo Institute of Material Technology and Engineering, Chinese Academy of Sciences, Ningbo, China
  - Material Sciences: AiGuo Wu
- Tohoku University, Sendai, Japan
  - Radiation Biology: Tetsuia Ono, Yoshihiko Uehara